Anatomical and Morphological Features of the Assimilation Apparatus and Haustoria of White Mistletoe Viscum album L. on the Host Plant in the Context of Its Semi-parasitic Lifestyle

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Abstract

In the article the anatomical structure of mistletoe stems, ways of its penetration into the host plant are provided. The anatomical features of the structure of mistletoe leaves are shown, the presence of drusen crystals in it, the number of conducting bundles are established. The absence of differentiation of the parenchyma into columnar and spongy is shown. The haustoria mistletoe contains chlorophyll. The differentiation of haustoria, their branching and the way of penetration into wood are shown. The formation of folds on the bark of the host plant has been shown when seeds or haustoria of mistletoe germinate. The mistletoe stem was also studied, the location of the conductive bundles was shown, as well as the formation of protective modifications of the host plant when damaged by a semi-parasite.

The analysis of chlorophyll content in Viscum album on different host plants was performed, and a quantitative comparison of Viscum album leaf parameters on different host plants was performed.

Key words: *Viscum album, semi-parasite, host plants, anatomy, haustoria*

Аннотация

В статье освещено анатомическое строение стеблей омелы, пути ее проникновения в растение-хозяина. Показаны анатомические особенности строения листьев омелы, установлено наличие в них кристаллов друзов, установлено количество проводящих пучков. Показано отсутствие дифференциации паренхимы на столбчатую и губчатую. Отмечено что густории омелы также содержат хлорофилл. Показана дифференциация гаусторий, их разветвления и способ проникновения в древесину. Показаны образования складок на коре растения-хозяина, когда прорастают семена или гаустории омелы. Также исследован стебель омелы, показано расположение проводящих пучков, а также образование защитных модификаций растения-хозяина при повреждении полупаразитом. Был осуществлен анализ на содержание хлорофиллов в Viscum album на разных растениях-хозяевах, и проведено количественное сравнение параметров листьев Viscum album на разных растениях-хозяевах.

Ключевые слова: *Viscum album, полупаразит, растения-хозяева, анатомия, гаусторий.*

Introduction

Viscum album is a species of semi-parasitic plants of the sandalwood family (*Santalaceae*), which is distributed almost throughout Europe and in Ukraine in particular (Ельпитифоров, Иваницкая, Малашук, 2017).

Among the deciduous plants, the orders *Fabales, Fagales, Lamiáles, Sapindáles, Rosales, Malvales* and *Malpighiales* predominate (Yelpitiforov, Klymenko, 2020). And the further, the more sincere this trophic series becomes. Mostly semi-parasite grows on old specimens. This is due to the fact that the transport system of the host plant is already formed and when the semi-parasite is affected, the tree can feed the "adapter" for a long time.

It inhabits the aboveground parts of deciduous, rarely coniferous trees. Perennial shrub (20-60 cm tall), mostly spherical (20-120 cm in diameter) with forked, bare, greenish-yellow, woody branches. Under the bark of trees, host plants penetrate and develop suckers (haustoria), growing deep into the trunk. Leaves opposite (3-6 cm long, 6-15 cm wide), evergreen, sessile, leathery, thick, yellow-green, elongated, obtuse at the apex, entire, glabrous. The flowers are yellow, inconspicuous, heterosexual, sessile, located 3-6 in the forks of the branches. Dioecious plants. Staminate flowers with simple, corolla, tubular, four-parted perianth, four stamens, sessile anthers, attached to the perianth leaves. Perianth of pistillate flowers with 3-4 separate crook; column

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absent, sessile, conical, lower ovary. Fruit - berry, white, spherical or short-oval (6-9 mm long, 5.5-9.5 mm wide) with a slightly depressed tip, one- or two-seeded (Hawksworth, Wiens, 1996).

According to our data, the plants of the species affect 50 host plants on the territory of the National Botanical Garden MM Grishko (Yelpitiforov, Klymenko, 2020), according to other data - 122 subspecies, varieties and hybrids that grow in Ukraine, including the Crimea. According to the latest data (Krasylenko, Gleb, Volutsa, 2019) in Ukraine, plants from other genera related to the genus *Viscum*, родів – *Arceuthobium oxycedri* (DC.) M.Bieb. and *Loranthus europaeus* Jacq., which inhabit junipers and oaks, respectively.

The aim of this research - the explored of the anatomical and morphological structures of mistletoe by the method of studying transverse sections of a leaf and plant shoots.

The study of the anatomical structure of plants of this genus, primarily the *Viscum coloratum* L., was carried out by researchers from Siberia and the Urals. The results of their work confirm the xenomorphic nature of the plant, and the results of this study of the shoots of *Viscum album* coincide with the previous anatomical and morphological studies of plants of this genus. This indicates that the host plant does not affect the anatomical and morphological structure of the hemiparasite shoot plant.

Materials and methods

Biological material was collected in the Kyiv region (24 km from Kyiv), 13.03.2020 and on the same day fresh cross-sections of leaves, haustoria with wood and apical buds of mistletoe were made with a blade. The host plant on which the semi-parasite grew was identified as Betula pendula, at least 20 years old. The choice of the plant is largely due to the fact that it is quite common in Ukraine, has soft wood and is often affected by semi-parasites. Collected 25 shoots of approximately the same age from different parts of the crown of three trees of the same species to determine if there was a difference in the structure of the semi-parasite depending on its location on the tree. The affected branches were collected from the lower, middle and upper parts of the crowns, with a total mass damage to the crown of more than 70%. Anatomical studies were performed using a light stereoscopic microscope STEMI 2000-C, photographs taken in the program Axio Vision.

The chlorophyll content of *Viscum album* on different host plants was also determined. The pigments were extracted from the plant material with 80% acetone and determined spectrophotometrically at λ = 663, 646, 470 (Lichtenthaller, 1987). The pigment content was calculated in mg/g of crude material. A quantitative comparison of *Viscum album* leaf parameters on different host plants was also performed using measurement statistics methods.

Results and discussion

Viscum album in the process of interaction "host-parasite" faces many problems, including overcoming the protective forces of the host and providing access to water resources of the host (Hawksworth, Wiens, 1996). Of great importance is the avoidance of mineral deficiencies, differences in the chemistry of the juice of the host (Glatzel, Geils, 2008). The anatomical structure of the organs of *Viscum album*, which contribute to the life of the plant and aimed at its development and distribution is largely due to these processes.

Provided that the development of *Viscum album* will operate without special obstacles, 2-3 year old sprout in cross section reaches 5-6 mm. In general, *Viscum album* live 10-12 years (Krasylenko, Gleb, Volutsa, 2019).

The cross section of the leaf (Fig. 1) indicates that the epidermis covering the leaf - single-layer, has a thick cuticle. It is possible, as in *Viscum coloratum* L. (Щёкина, Крылов, 2011), it is

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covered with epicular wax. The stomata are slightly raised above the epidermis or placed flush with the epidermocytes, in contrast to the epidermis, for example, *Viscum coloratum.*

Conducting bundles can be up to 15 pieces depending on the size of the leaf.

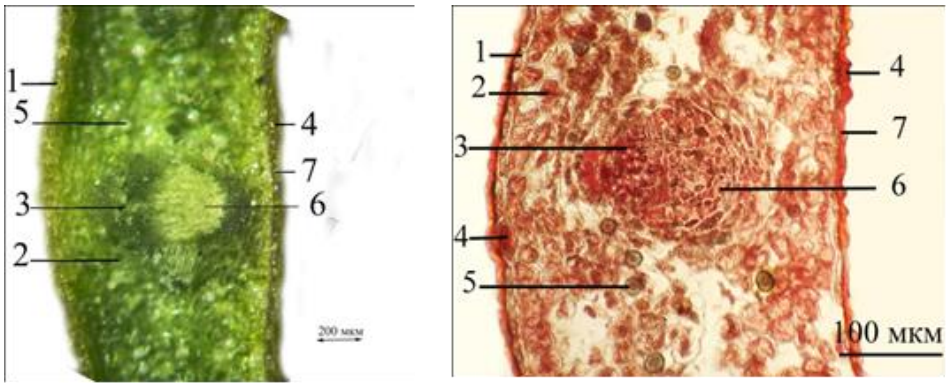

Fig. 1. *Cross section of Viscum album leaf: 1) abaxial epidermis with a thick cuticle, 2) mesophile, 3) xylem, 4) stomata, 5) inclusion of calcium oxalate, 6) phloem, 7) adaxial epidermis with a thick cuticle*

In white mistletoe spongy, undifferentiated structure of the mesophile. Mistletoe leaves do not develop the typical bipolar differentiation into columnar and spongy parenchyma. Their tissue remains at the meristematic stage with a further increase in thickness and length until next spring, which is also confirmed by the literature data (Calder, Bernhardt, 1983).

The letter contains inclusions of calcium oxalates CaC_2O_4 in the form of druse, which are derivatives of oxalic acid (Раздорский, 1949). They are present only in the leaves of the semi-parasite, the trunk and haustoria do not contain them. Some authors (Heide-Jørgensen, 1989) explain the accumulation of these elements by the fact that they are actively used by the semi-parasite in the regulation of osmosis and stomatal control. Although calcium may act as an antagonist of potassium and phosphorus, which in comparison with the host in the tissues of the semi-parasite more than in the tissues of the host (Ельпитифоров, Иваницкая, Малашук, 2017).

Interesting is the fact that the haustoria of the studied plants contain chlorophyll (Fig. 2), which can be seen under magnification under a microscope.

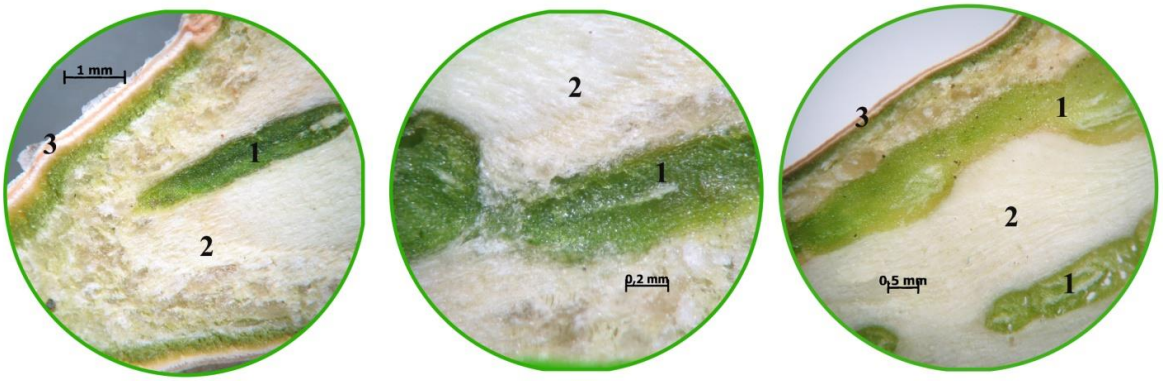

Fig. 2. Haustoria of the semi-parasite Viscum album, which are inside the host plant Betula pendula*
1 - haustoria, 2 - wood of the host plant Betula pendula, 3 - bark of the host plant Betula pendula
** Anatomical sections of green haustoria are shown under different magnifications*

During germination, the semi-parasite does not cut into the wood of the host. The seed secretes special enzymes (lectins or thionines) (Bhandari, Mukerji, 1993), which do not germinate in the wood, but cause the wood to grow around the seed, stimulating the protective mechanisms of the host plants. Haustoria cells of *Viscum album* go deep into the periderm perpendicular to the stem of the host plant. From the growth in the horizontal direction the first haustoria, or rizons.

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From them the branched system of accompanying haustoria is formed. Haustoria radially, through the bast and cambium, penetrate to the secondary xylem and propagate parallel to the fibers (Fig. 3.)

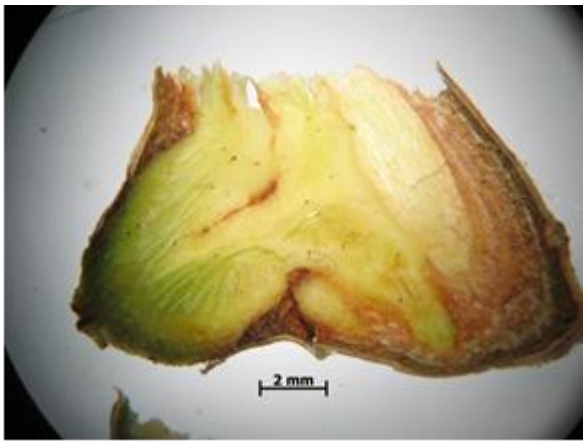

Fig. 3. *Germination and branching of Viscum album, inside the host plant Betula pendula (green is Viscum album)*

Some of the cells connect with the cells of the host vessels and due to the higher osmotic potential in the xylem of the semi-parasite *Viscum album* receives water with dissolved minerals from the host. That is why it is not surprising that there is a higher rate of transpiration in mistletoe than in the host plant (Тарaн. 2007).

After infection, the developing mistletoe begins to use water from the host system. Some species of mistletoe, especially from the family *Loranthaceae*, are able to almost completely use the substances of the host phloem (*Bowie and Ward 2004*), but in *Viscum album* it occurs in part and depends on the host plant, which is also characteristic of the interaction in system *Viscum album-Betula pendula*. Thus, for the *Viscum album*, this form of existence can be considered semi-parasitic, even if it provides photosynthesis is incomplete. After the initial branching, haustoria grow together with the host stem due to the intercalary meristem. Haustoria form a kind of strands, possibly with the release of specific adhesives, most likely lectins or thionines (Bhandari, Mukerji, 1993) to dissolve the wood of the host plant (Fig. 4).

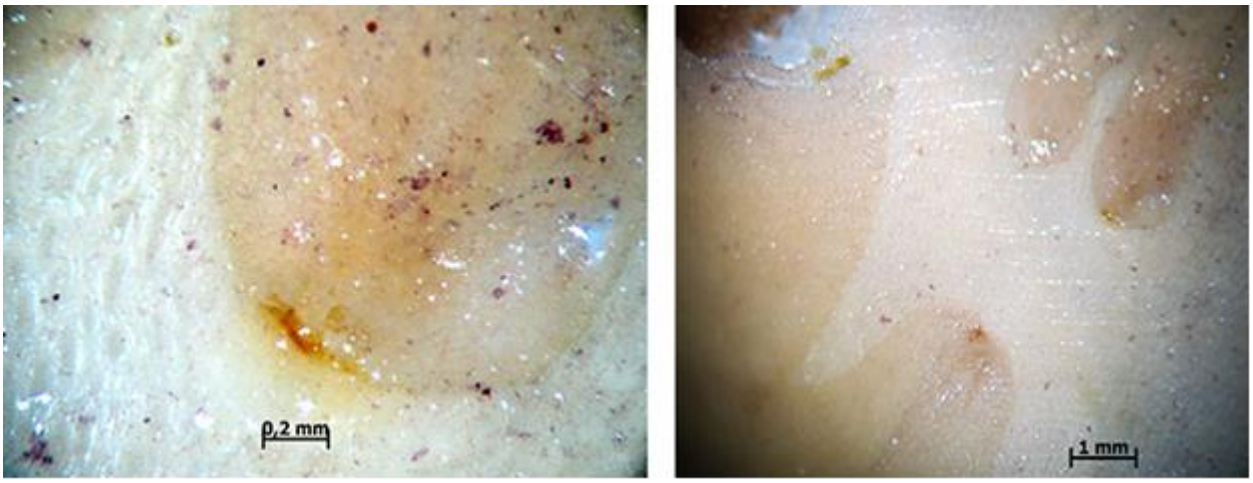

Fig. 4. *Apical points of Viscum album haustoria, inside the host plant Betula pendula*

On average, the crown of the *Viscum album* bush corresponds in size to its haustoric system (Hawksworth, Wiens, 1996).

No less interesting is the formation of folds of bark on the trunks of the semi-parasite, which are especially visible after the second year of life (Fig. 5). The same folds of bark are in the host plant, which is obviously a defense mechanism, the ability to increase its surface area.

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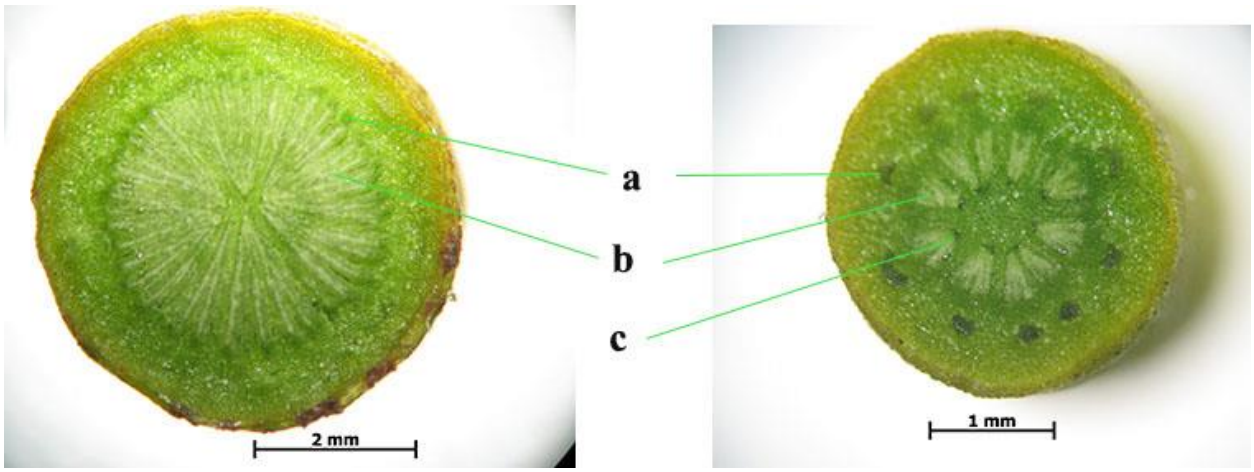
Fig. 5. *The formation of folds of bark on the trunk of Viscum album, adjacent to the place of ingrowth into the host plant*

If you take an annual shoot of the plant and study its anatomical structure, the cross section shows the epidermis, which it is covered. The epidermis is single-layered, covered with epicuticular wax, like a leaf. Next is a spongy mesophile, which houses the bundles of phloem, and below - the primary core rays with the primary xylem (Fig. 6).



I II

Fig. 6. *Cross section of an annual shoot Viscum album. (I) closer to the apical bud, II - farther from the apical bud. a - phloem bundles, c - primary core rays with primary xylem (c)*

However, in the cross section of the stem closer to the haustoria (Fig. 6 (II)), the xylem is almost invisible, and the heart-shaped rays penetrate the stem almost to the center. At the apex of the shoots, two germinal leaves are formed, which in cross section are somewhat similar to open stomata and have a bean shape, and closer to the stem, below the apical bud, have a radial structure (Fig. 7).

Betula pendula is able to secrete protective substances, which it tries to separate the tissue from the intervention of the semi-parasite, which is visible on the brown transverse lines, most likely birch tar. However, this does not prevent the semi-parasite from penetrating into the tissues of the host plant by haustoria.

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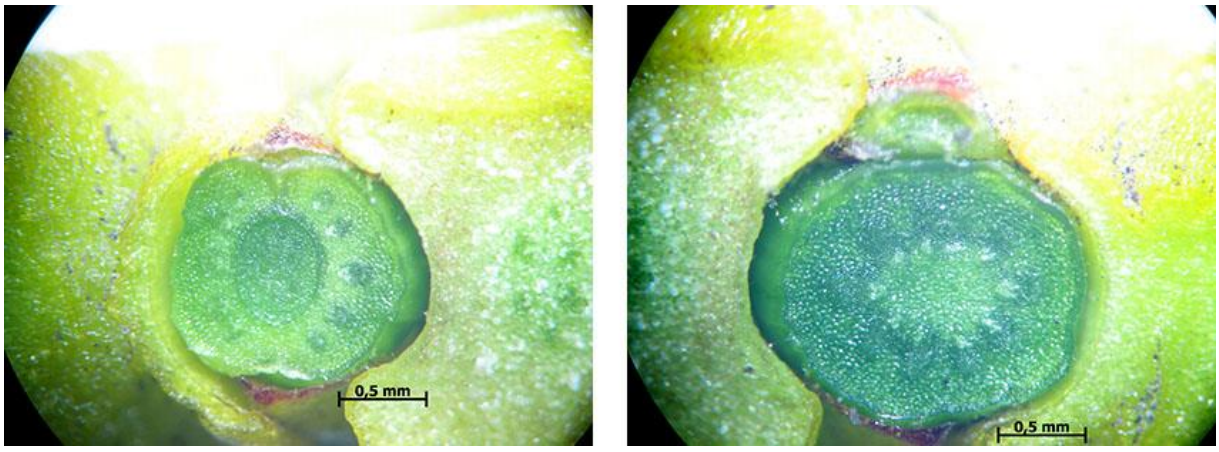

a **b**

Fig. 7. *Cross section of apical buds Viscum album*
a - closer to the apical bud, b - farther from the apical bud

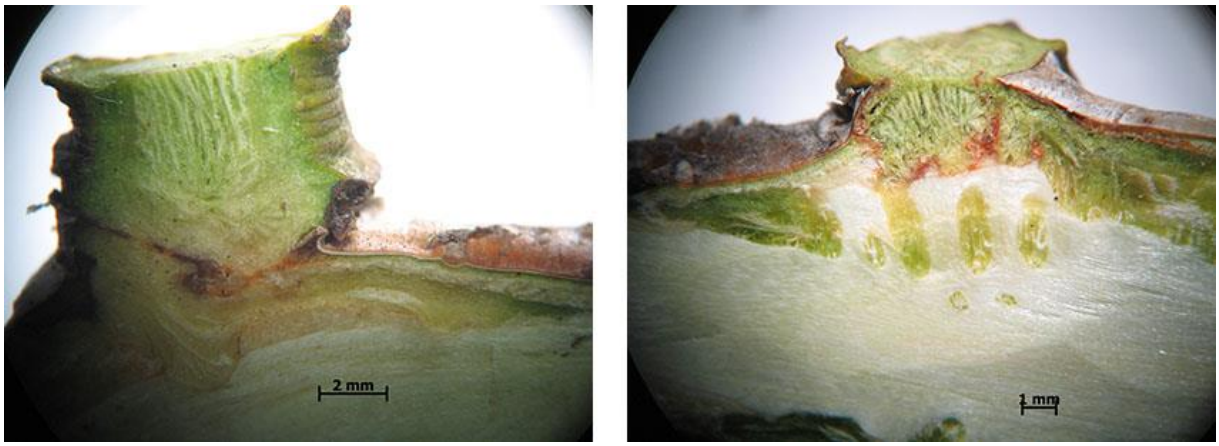

Fig. 8. *Formation of a protective barrier by the host plant when inhabiting its Viscum album*

It should also be noted the fragility of the branches of the semi-parasite. With a large snow cover or mass growth on the branches of the host plant, the semi-parasite can drop part of the unwanted green mass along with the seeds, thus preserving the haustoria.

White mistletoe settles on 50 host plants in the NBS M.M. Grishko, and the quantitative indicators of its assimilation apparatus are quite different, if we talk about the different hosts on which it grows (Fig.8). Thus, the width and length of the leaf blade of *Viscum album* among all studied plants is the largest on *Aesculus hippocastanum*, and the smallest - on - *Crataegus monogyna*, which can correlate with the size of the leaves of these plants (Table 1).

Table 1*. Quantitative indicators of length and width of Viscum album leaves on different host plants*

Host plants	Width±σ	Length± σ
Malus **domestica**	16,03±3,16a	57,25±12,39b
Aesculus hippocastanum	16,68±3,55a	67,42±8,14a
Crataegus monogyna	10,67±1,83b	44,70±7,98c
Acer *negundo*	10,50±2,22b	65,30±8,47ab
Robinia pseudoacacia	10,80±1,03b	47,30±2,67c

* The same letters indicate the absence of a significant difference between different hosts (P≤0,05).

The width of the mistletoe leaf blade on *Malus domestica* and *Aesculus hippocastanum* is significantly greater than that on other studied hosts.

Viscum album from *Aesculus hippocastanum* morphometrically differs most from *Viscum album* on other host plants.

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It should also be noted that the number of pigments in *Viscum album* is the same on different hosts, but *Aesculus hippocastanum* differs slightly more in the ratio of pigments. Perhaps this is due to the fact that the semi-parasite as a whole uses this plant as a host not so long ago as other plants (Table 2).

***Table 2**. The presence of pigments in the leaf Viscum album, which grows on the studied plants, and their ratio*

	A chla	**A chl b**	**A car**	**chla/chl b**	**(Chla+chlb)/car**
Malus **domestica**	1,46±0,46a	0,30±0,05a	0,52±0,22a	4,82±0,92ab	3,54±0,49ab
Aesculus hippocastanum	1,10±0,26a	0,30±0,03a	0,35±0,10a	3,64±0,63b	4,10±0,40a
Crataegus monogyna	1,67±0,32a	0,26±0,05a	0,64±0,13a	6,42±0,88a	3,04±0,27b
Acer *negundo*	0,98±0,25a	0,25±0,02a	0,36±0,13a	3,83±0,63bc	3,65±0,55ab
Robinia pseudoacacia	1,61±0,26a	0,28±0,01a	0,61±0,07a	5,82±0,9ac	3,10±0,06b

Today, the only effective way to combat *Viscum album* is sanitary felling, topping (or continuous removal of the crown) and continuous felling.

Morphologically, the leaves and shoots of *Viscum album* at the apex of the crown are larger; anatomically, there are practically no differences between them. Likewise, there is little or no difference between semi-parasitic plants on different host plants.

Conclusions

In general, the structure of the shoot, leaves and haustoria of *Viscum album*, as well as the presence of chlorophyll contributes to a semi-parasitic lifestyle, although some scientists claim that the plant may be a complete parasite. In general, tissues are characterized by xenomorphism, the presence of wax substances and a significant acceleration of transpiration processes. Penetrating into the host's host system, the *Viscum album* merges with its xylem to get water and possibly some organic matter. Thus, we can talk about chemical means of struggle, such as herbicides, only in the context of the not evergreen of the host. Mechanical means of regulating the number of *Viscum album* are effective provided that the affected areas are completely excised. Pruned haustoria after some time are restored and branched, forming new plants *Viscum album.*

Bibliography

1. Bhandari, N. N. & Mukerji, K. G. (1993). *The haustorium*. - Wiley. New York.
2. Calder M & Bernhardt, P. (1983). *The biology of Mistletoes*. Academic Press Australia, Sydney.
3. Yelpitiforov, E., Klymenko, Y. (2020). European mistletoe (Víscum álbum L.) in national botanical garden M.M. Grushko NAS of Ukraine: an overview of its distribution and hosts, - Scientific journal "*Sciencerise:biological science*", - №3(24), - 2020, 24-28.
4. Glatzel, G., Geils, B.W. (2008). Mistletoe ecophysiology: host–parasite interactions. *Botany*, 87(1): 10 – 15.
5. Hawksworth, F.G., and Wiens, D. (1996). *Dwarf mistletoes: biology, pathology and systematics*. Agriculture Handbook, 709. USDA Forest Service, Washington D.C. p. 410.
6. Heide-Jørgensen, H S. (1989). *Development and ultrastructure of the haustorium of Viscum minimum* Harvey. I. The adhesive disk. - Can. J. Bot. 67: 1161-1173.
7. Issakainen, J., Pihlaja, K. & Kasvi A. (2019). Misteli on vakiintumassa Suomeen. *Lutukka*, 2019, 35. vsk, nro 1, 8-23.
8. Krasylenko, Yu. A., Gleb, R.Yu., Volutsa, O.D. (2019). *Loranthus europaeus (Loranthaceae*) in Ukraine: an overview of distribution patterns and hosts, Ukr. Bot. J. 2019, 76(5): 406–417
9. Lichtenthaller, H.K. (1987). Chlorophylls and carotenoids, pigments of photosynthetic biomembranes. *Methods in Enzymology*. 148, 350–382
10. Mathiasen, R.L., Nickrent, D.L., Shaw, D.C., Watson, D.M. (2008). Mistletoes: pathology, systematics, ecology, and management. *Plant Disease*, 92(7): 988 – 1006. https://doi.org/10.1094/PDIS-92-7-0988
11. Watson, D.M. (2001). Mistletoe – a keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics*, 32 (1): 219 – 249. https://doi.org./10/1146/annurev.ecolsys.32.081501.114024
12. Булгакова, Т.О. (1969). Омела та її рослини-господарі в дендропарку «Олександрія» АН УРСР // *Інтродукція деяких екзотів і політомічний метод їх визначення*.— К.: Наук. думка, 49–50.

* *Kontaktinis asmuo / Corresponding author*

13. Ельпитифоров, Е.Н., Иваницкая, Б.А., Малашук, Е.В. (2017). Сравнительная оценка содержания химических елементов *Viscum album* L. и *Viscum album* subsp. *Austriacum* (wiesb.) Vollmann // Львів: «Науковий вісник НЛТУ», №27 (5), 93-97
14. Раздорский, В.Ф. (1949). *Анатомия растений*. – М.: Советская наука. – 524 с.
15. Таран, Н. Ю. (2007). *Фізіологічне обґрунтування методів профілактики розповсюдження та боротьби з омелою білою у лісопаркових ландшафтах* / Таран Н.Ю., Бацманова Л. М., Мелешко А. О., Улинець В.З., Лукаш О.В.— К.: Ленвіт.
16. Щёкина, В.В., Крылов, А.В. (2011). Эколого-анатомические адаптации в строении листа омелы окрашенной *Viscum Coloratum* (kom.) Nakai Дальневосточный государственный аграрный университет: – №3 (19), 36-40

Анатомо-морфологические особенности ассимиляционного аппарата и гаусторий омелы белой Viscum album L. на растении-хозяине в контексте ее полупаразитического способа жизни

(Получено в сентябре 2021 г.; отдано в печать в феврале 2022 г.; доступ в интернете с 6 мая 2022 г.)

Резюме

В контексте все большей экспансии Viscum album в Украине и в Европе в том числе, ботаников все больше интересует строение растения-полупаразита, а также механизмы взаимодействия «хозяин-полупаразит». Это растение охватывает все больше «хозяев», поэтому актуальными становятся механизмы их взаимодействия. На генетическом уровне проводилось достаточно много исследований, в том числе и в Украине, но ассимиляционный аппарат и его анатомо-морфологическое строение показаны в литературе довольно слабо.

В общей сложности Viscum album живут 10-12 лет, но первые годы полупаразита протекают внутри «хозяина». Здесь растение развивается и разрастается гаусториями, в которых несомненно есть хлорофилл. Побеги и листья Viscum album с достаточно толстой эпидермой, что указывает на ксероморфность полупаразита. Мезофилл листка недифференцированный, а у побега нет полости внутри, все заполнено паренхимой.

Отмечено также наличие оксалатов кальция, но только в листьях, ствол и побеги их не содержат. Видимо, это связано с тем, что CaC2O4 активно используется в регуляции осмоса и контроля устьиц. Хотя, возможно, кальций работает как антагонист фосфора и калия. Этих элементов в тканях полупаразита больше чем в тканях растения-хозяина, как показывают наши предыдущие исследования.

Модель прорастания гаусторий полупаразита не такая, как у других растений. Полупаразит не прорастает сам, он стимулирует нарастание тканей хозяина вокруг гаустории, используя защитные механизмы растения и получая от него воду благодаря более высокому осмотическому потенциалу. Именно поэтому открытым остается вопрос транспирации Viscum album, ведь ее показатели намного выше, чем у растений, на которых она поселяется.

В среднем, крона куста соответствует его гаусториальной системе.

Растение-хазяин использует механизмы защиты от гаусторий полупаразита, в виде наростания тканей и образования складок, но в данном случае эти механизмы работают против него самого, увеличивая площадь разрастания гаусторий.

В случае, если полупаразит набирает большую массу, чем может выдержать хозяин, Viscum album может сбрасывать часть кроны вместе с семенами, разрастаясь внутри древесины благодаря гаусториям.

Морфологические показатели листьев Viscum album связаны с размером листьев растений-хозяев и расположения в кроне, других тенденций не отмечено, как и в показателях пигментов.

Сегодня возможны наиболее рациональные способы борьбы с Viscum album - это топинг, санитарные и сплошные рубки.

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